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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	205032000400	*Total Pages*	34
Inventors			
Li-Wei Hsu and Su-Chen Chang			
Express Mail Label No.	EL628459416US		

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231**

1. [X] Fee Transmittal Form *(Submit an original, and a duplicate for fee processing)*
2. [X] Specification [*Total Pages* 12] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 USC 113)* [*Total Sheets* 11]
4. [X] Oath or Declaration [*Total Pages* 3]
   a. [X] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63(d) *(for continuation/divisional with Box 17 completed)* ***[Note Box 5 below]***
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1 33(b)
5. [X] Incorporation By Reference *(useable if Box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. [ ] Microfiche Computer Program *(Appendix)*
7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
   b. [ ] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [X] Assignment Papers (cover sheet & document(s))
9. [ ] 37 CFR 3.73(b) Statement [ ] Power of Attorney *(when there is an assignee)*
10. [ ] English Translation Document *(if applicable)*
11. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
12. [ ] Preliminary Amendment
13. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
14. [X] Small Entity Statement(s) [ ] Statement filed in prior application, Status still proper and desired
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16. [ ] .....

17. If a CONTINUING APPLICATION, *check appropriate box and supply the requisite information:*

*[ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: *

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sd-19115

☒ If a paper is untimely filed in the above-referenced application by applicant or his/her representative, the Assistant Commissioner is hereby petitioned under 37 C.F.R. § 1.136(a) for the minimum extension of time required to make said paper timely. In the event a petition for extension of time is made under the provisions of this paragraph, the Assistant Commissioner is hereby requested to charge any fee required under 37 C.F.R. § 1.17(a)-(d) to **Deposit Account No. 03-1952**. However, the Assistant Commissioner is **NOT** authorized to charge the cost of the issue fee to the Deposit Account.

The filing fee has been calculated as follows:

FOR	NUMBER FILED	NUMBER EXTRA	RATE	CALCULATIONS
TOTAL CLAIMS	33 - 20 =	13	x $18.00	$234.00
INDEPENDENT CLAIMS	2 - 3 =	0	x $80.00	$0
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$270.00
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Dated: November 7, 2000

Respectfully submitted,

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Applicant/Patentee: Li-Wei Hsu and Su-Chen Chang	Docket No.: 205032000400
Serial No./Patent No.: Not Assigned	
Filed on/Issued: Filed Herewith	
For: Novel method for the screening of extract of plant for active ingredients	

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
37 C.F.R. §§ 1.9(f) AND 1.27(c) — SMALL BUSINESS CONCERN

I hereby declare that I am

☑ the owner of the small business concern identified below:
☐ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: Advanced Gene Technology

ADDRESS OF CONCERN: No. 2-1, 7th Road, Taichung Industrial Park, Taichung City, Taiwan, R.O.C.

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 121.12, and reproduced in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled Novel method for the screening of extract of plant for active ingredients by Li-Wei Hsu and Su-Chen Chang, described in

☑ the specification filed herewith with title as listed above.
☐ the application identified above.
☐ the patent identified above.

If the rights held by the above identified business concern are not exclusive, each individual, concern or organization having rights in the invention must file separate verified statements averring to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d), or a nonprofit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization having any rights in the invention is listed below:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Fu-Tao Lin
TITLE OF PERSON IF OTHER THAN OWNER: President
ADDRESS OF PERSON SIGNING: No. 2-1, 7th Road, Taichung Industrial Park, Taichung City, Taiwan, R.O.C.

SIGNATURE: _____ DATE: 25, 10, 00

Invention Title

Novel Method For The Screening OF Extract Of Plant For Active Ingredients

Technical Field of the Invention

The present invention relates to the application of high-density gridding technology for the screening of the fractions of an extract of plant for biologically active ingredients of interest.

Background of the Invention

Plants, especially herbs, are natural sources of biologically active ingredients that have a pharmacological or therapeutic effect in animals. Many drugs or their precursors are isolated from plants. Normally, it is laborious to isolate a biologically active ingredient in a purified form from plant or its extract by applying conventional techniques, such as extraction, precipitation, centrifugation and complicated chromatographic technology. So far there is not a simple method for the screening of plant or its extract to select biologically active ingredients in a rapid manner.

Recently, the high-density gridding technology has been used in the art for qualitatively or quantitatively detecting the presence of a target material in biological samples. The high-density gridding technology immobilizes arrays of samples in a small or even tiny volume on a gridding surface of a solid support. In this technology, the biological samples suspected of containing target material are fixed or immobilized on a solid support, a labeled probe that can hybridize with or conjugate to the target material is added to the solid support, the hybridized or conjugated solid support is processed, imaged and analyzed, and the candidate target material that specifically interacts with the labeled probe can be quickly screened and selected.

However, so far the target material that can be successfully detected on a solid support by applying the high-density gridding technology is limited to several macromolecules, such as nucleic acid fragments/sequences or peptides or proteins. For instance, USP 6,004,755 disclosed a method for hybridization assay, comprising contacting an array of probe molecules stably associated with the surface of a solid support with an end labeled target nucleic acid sample under hybridization conditions for producing a hybridization pattern. USP 6,040,138 disclosed a method of hybridizing a nucleic acid sample to a high density array of oligonucleotide probes, in which the oligonucleotide

probes in the high density array are complementary to the subsequences of target nucleic acids in the nucleic acid sample. USP 6,087,103 disclosed a method of screening a target for ligand binding, comprising using a tagged array of protein ligands bound to a solid support. It is not taught or suggested in the prior art or the state of the art that biologically active small molecules can be successfully screened and selected by using the high-density gridding technology.

There is a demand in the development of a rapid method for the screening of plant or its extract for biologically active small molecular components in a massive amount by using a simple process. In addition, to meet a technical requirement for the screening, there is also a demand in the development of a simple kit for the screening of plant or its extract for biologically active small molecular components.

Summary of the Invention

The present invention relates to a novel method for the screening of an extract of plant for biologically active ingredients, comprising allocating the components fractionated from a crude extract of plant on a solid support, adding labeled target as a probe onto the surface of gridded area of the solid support, properly incubating the solid support for achieving specific interaction, then detecting the expected signals, and selecting and recovering the biologically active ingredients corresponding to the signals.

One embodiment of the present invention relates to the use of said novel method for obtaining a biologically active compound in a purified form from an extract of *Carthamus tinctorius L* that can specifically bind to a platelet membrane receptor protein gpIIb/IIIa.

Brief Description of the Drawings

Figure 1A shows the HPLC elution profile of an extract of *Carthamus tinctorius L* in 120 minutes.

Figure 1B shows the binding profile of the eluted samples shown in Figure 1A to the protein gpIIb/IIIa that was detected by the absorption at a wavelength of 405 nm.

Figure 2A shows the HPLC elution profile of a sample pool collected from the fractions No. 25 to 40 shown in Figure 1A in 20 minutes.

Figure 2B shows the binding profile of the eluted samples shown in Figure 2A to the protein gpIIb/IIIa that was detected by the absorption at a wavelength of 405 nm.

Figure 3 shows the HPLC elution profile of a sample pool collected from the fractions No. 5 and 6 shown in Figure 2A in 15 minutes, indicating that there is a single peak with retention time of 10.7 minutes which shows the strongest binding activity to the protein gpIIb/IIIa.

Figure 4A shows the molecular weight 268 gm/mole of the compound obtained from the profile of Figure 3 that was detected by the electrospray mass spectrum.

Figure 4B shows the presence of sodium salt and oligomers (2-mer to 7-mer) of the compound obtained from the profile of Figure 3.

Figure 5 shows the binding curve of the compound obtained from the profile of Figure 3 with the protein gpIIb/IIIa, indicating that the binding dramatically increased up to 80 % of the maximum binding when the concentration of the compound is below 10 μg/ml.

Figure 6A shows a dose response of the compound obtained from the profile of Figure 3 in the inhibition of platelet aggregation, indicating that the maximum inhibition activity is about 85 % when the concentration of the compound is from 10 to 15 μg/ml blood.

Figure 6B shows the inhibition activity on platelet aggregation in a time course manner when the concentration of the compound obtained from the profile of Figure 3 is 17.4 μg/ml, indicating that about 85 % of the maximum inhibition activity occurs at a period from 14^{th} to 16^{th} minutes.

Figure 7A shows the inhibition of thrombosis formation *in vivo* in mesenteric vein of rats by administering the compound (400 μg/100 μl/rat) obtained from the profile of Figure 3 in a time course manner.

Figure 7B shows a dose response of the compound obtained from the profile of Figure 3 in the inhibition of thrombosis formation *in vivo* in mesenteric vein of rats by intravenous injection.

Figure 7C shows the inhibition of thrombosis formation *in vivo* in mesenteric vein of

rats by oral administration of the compound (4mg/rat) obtained from the profile of Figure 3 in a time course manner.

Detailed Description of the Invention

The present invention provides a rapid method for the screening of an extract of plant for biologically active ingredients. The method comprises fractionating a crude extract of plant, allocating the components in each fraction on a solid support, adding labeled target as a probe onto the solid support, incubating the solid support to allow the specific binding between biologically active ingredients of interest in the crude extract and the added probe, and then detecting and selecting the biologically active ingredients of interest.

It is well known in the art that plants, especially herbs, are treasures of natural sources for new drug discovery. Isolation of biologically active ingredients in a purified form from plants by using the conventional techniques is laborious and time-consuming.

The high-density gridding technology has been used in the art for detecting a target material of interest in samples, which involves immobilizing arrays of samples in a small or even tiny volume on a gridding surface of a solid support. It is an advantage that the high-density gridding technology can perform hundreds or more of different experiments as in individual test tubes at the same time. By this technology, samples suspected of containing a target material of interest are fixed or immobilized on a solid support, a labeled probe that can hybridize with or conjugate to the target material is added to the solid support, the hybridized or conjugated solid support is processed, imaged and analyzed, and the candidate target material that specifically interacts with the labeled probe can be quickly detected. In practice, the high-density gridding technology has been used in the field of diagnosis, drug selection, discovery of therapeutic targets, and determination of pharmacological mechanism.

The target materials that can be successfully detected in the art by applying the high-density gridding technology are, however, limited to several macromolecules, such as fragments of nucleic acids or proteins. It is not known, taught or suggested in the art that small molecules exhibiting a biological activity of interest can be successfully detected and selected by applying the high-density gridding technology.

It is a discovery of the present invention that biologically active small molecules can be detected and isolated in a purified form from an extract of plant by applying the

high-density gridding technology. According to the method of the present invention, a crude extract of plant is fractionated with the aid of chromatography, the components in individual fractions are immobilized on the surface of a gridding solid support, the solid support is then hybridized with a labeled target, the sites on the solid support containing the biologically active small molecules interacting with the labeled target are detected, and the biologically active small molecule is then recovered, isolated and finally purified.

In one embodiment of the present invention, an extract of herb was fractionated by HPLC in the beginning. Individual fractions are allocated on the surface of a gridding solid support. A labeled protein was added to the surface of the solid support for hybridization. The unbound protein was stripped off thereafter. The candidate fractions showing a signal of binding to the labeled protein were selected, and the previous steps are repeated until a single ingredient interacting with the labeled protein was obtained.

In one preferred embodiment of the present invention, an extract of *Carthamus tinctorius L* was fractionated by HPLC. Individual fractions were allocated on a plastic plate. A labeled platelet membrane receptor protein gpIIb/IIIa, an important factor capable of inhibiting platelet aggregation *in vitro* and thrombosis formation *in vivo*, was added to the plastic plate for binding. The unbound gpIIb/IIIa was stripped off. The candidate fractions showing a signal of binding to the labeled gpIIb/IIIa were selected, and the previous steps are repeated until a single ingredient interacting with the labeled gpIIb/IIIa was obtained.

The method of the present invention will be more efficient if integral automatic instruments for sampling, high-density arraying and detecting are used.

The present invention reveals the following advantages and improvement in effect: (i) the sample volume for screening can be minimized to a micro- or even pico-liter level when the samples are spotted onto the surface of a solid support, (ii) the presence of selected small molecules that directly interact with the labeled target clearly demonstrate that there is a specific binding between the small molecules and labeled target, and the screened small molecules may exhibit the desired pharmacological function, (iii) the solid support spotted with fractions of an extract of plant can be used as a chip of a specific plant, for instance as a herbal chip, that can be universally used as a platform for screening for a biologically active ingredient of interest, and (iv) the screening process can be preformed within a high density-spotted solid area

instead of dealing with a large amount of reactions in test tubes.

The present invention will be further illustrated by the following examples; however, it should be noted that the present invention is not limited to the following examples.

Examples

Example 1

Five gram of *Carthamus tinctorius L* (purchased from Uni Chinese Herb Store, Taichung, Taiwan) was extracted with methanol (40 ml) by regular blending. The extract was concentrated to a final volume of 8 ml. For conducting HPLC (Shimadzu 10-AT, Japan) analysis, the concentrated extract (100 μl) was injected into ODS-gel ODS 80TM (4.6 mm × 25 cm, TOSOH, Japan) column. The concentrated extract was first eluted with water for 5 minutes, followed by elution with an ethanol-water eluant with a linear increase of ethanol concentration from 0 to 70 % (v/v) within 105 minutes. In the next 5 minutes for elution, the concentration of ethanol in the eluant was increased to 100 % (v/v). The eluted samples were detected at a wavelength of 254 nm and were collected every 0.5 ml. The elution profile was shown in Figure 1A.

For the target-binding assay, the platelet membrane receptor protein gpIIb/IIIa was purified from platelets, and its purity was determined by SDS-PAGE and silver staining. The purified protein gpIIb/IIIa was labeled with biotin as described in the conventional protocol. The 120 collected samples were individually coated onto a 384-well plastic plate. The biotin-labeled protein gpIIb/IIIa was added to the sample-coated plastic plate and was incubated with the coated samples at room temperature for 30 minutes. Each well was then washed with TBST buffer for 3 times, to which the extravidin-conjugated alkaline phosphatase was added, and each well was further incubated for 30 minutes. The previous washing step was repeated and each well was then colored with substrate p-nitrophenyl phosphate. The absorption for each well at a wavelength of 405 nm was determined by an automatic ELISA reader (Dynax). Figure 1B shows the OD_{405nm} value of the samples corresponding to those in Figure 1A, indicating their capacity of binding to the protein gpIIb/IIIa.

Example 2

The fractions corresponding to No. 25 to 40 in Figure 1A were pooled together, and were injected into the column and analyzed by HPLC in a manner similar to that

described in Example 1. The ethanol-water elution was changed to a 20-minute program with a linear increase of ethanol concentration from 20 to 30 %. The elution profile is shown in Figure 2A.

The target protein gpIIb/IIIa-binding assay was performed in a way as that described in Example 1. The result indicating the capacity of gpIIb/IIIa binding for each fraction is shown in Figure 2B.

Example 3

The fraction 5 and 6 shown in Figure 2A were pooled together, on which there was run a third round HPLC analysis with an isocratic eluant of 9 % ethanol and 91 % water for 20 minutes. A single peak with retention time of 10.7 minute showing the strongest binding activity is obtained. The refining profile is shown in Figure 3.

The compound collected from the profile of Figure 3 was dried, and its molecular weight (MW) was determined by the electrospray mass spectrum. As shown in Figure 4A, the major peak was determined to be an m/z of 268.04 with a standard deviation of 0.11. Another 289.9 m/z paired peak was also determined by approximately 21.9 m/z apart. It was proposed to be a sodium salt of the major peak. Furthermore, a number of related, multiply-charged and paired peaks (535.6; 802.1; 1069.7; 1357.9; 1604.4; and 1871.1 m/z) were also determined (Figure 4B). Multiply-charged electrospray is often determined for biopolymers, and those peaks shown in Figure 4B were exactly correspondent to dimmer to 7-mer, respectively. A paired signal by approximately 22 m/z apart associated with each multiplied peak was proposed to be its conjugated sodium salt. In conclusion, the isolated compound has molecular weight of 268 gm/mole and exhibits a polymer-forming capacity.

Example 4

The profile shown in Figure 3 was used as a standard for the characterization of each batch preparation of the compound. The purified compound was collected, dried and used to verify its gpIIb/IIIa binding capacity. For conducting the binding assay, the purified compound was prepared in an aqueous solution, and a serial dilution for forming a final concentration of 0 to 50 μg/ml is made. Each tested solution contained a different amount of the compound, and was spotted on a flat well of a plastic 96-well plate. The labeled protein gpIIb/IIIa was then added into each tested well, and the coloring process was performed in a way as previously described. The binding curve

was shown in Figure 5. It was observed that the binding dramatically increased up to 80 % of the maximal binding with the concentration below 10 μg/ml.

Example 5

To assay the platelet aggregation-inhibiting activity of the compound, a regular ADP-activated platelet aggregation assay was performed. The reagents used for conducting the assay were purchased from Sigma. Figure 6A showed a dose response of the compound in the inhibition of platelet aggregation. The maximal inhibition activity was about 85 %, which occurs at the concentration of the compound from 10 to 15 μg/ml blood. By using the compound with the concentration of 17.4 μg/ml for determining the inhibition activity on platelet aggregation in a time course manner, Figure 6B revealed that the maximal inhibition activity was about 85 % occurring at the time from the 14^{th} to 16^{th} minutes.

Example 6

The thrombosis formation *in vivo* in mesenteric vein of rats was used for evaluating an effect of the compound on the inhibition of venous thrombosis. In brief, groups of 3 male Wistar-derived rats, each weighing 60±10 gm, were used for each dosage test. The rats were anesthetized with phenobarbital sodium (50 mg/kg, i.p.), and paralyzed with succinylcholine chloride (2 mg/rat, i.p.). A mesenteric loop and vein was then exposed and mounted on a constructed platform. The exposed area was superfused with a normal saline at 37 ℃, except during electrode placement and stimulation. With the aid of a dissecting microscope and micromanipulator, a monopolar platinum electrode was brought into and contact with the vein. Thrombus formation was initiated by the application of a single square wave electrical pulse (1000PPS, 100V, 300ms) supplied from a grass S-44 stimulator. The formation of thrombus was then observed through the microscope-calibrated ocular lens. The relative venous occlusion (a measured degree of thrombus formation) was determined as a percentage of venous diameter (0.36 to 0.38mm) and was recorded at 10 seconds (baseline control score) and at 1 minute interval for 20 minutes. The tested compound or vehicle was administered i.v. for 5 minutes before conducting the electrical stimulation of mesenteric vein. In the vehicle-treated animals, the 20 interval recorded values were averaged, which attains a value ranging from 45 to 55% of the interval venous diameter. Consequently, the antithrombic activity of tested compound was calculated as % of inhibition relative to the vehicle-treated control animals. If a significant inhibition (>30%) was observed in 3 animals, an ED_{30}±SEM was determined by

linear regression using 3 animals per dose level. At each time point, a paired Student's *t* test was applied for the statistical analysis for comparison of the vehicle treatment group with the tested compound group with a significance referred to as $^{*}P<0.05$ and $^{**}P<0.01$.

In Figure 7A, the compound exhibiting an antithrombic activity with a concentration of 400 μg/rat revealed significance relative to the vehicle treated group at 7, 11,12, 13, 14, 15, 16, 17, and 19 minutes after inducing an electric stimulation. The antithrombic activity of the tested compound in a dose response manner in a range of 25, 50, 100, 200 and 400 μg/rat was shown in Figure 7B.

For evaluating the antithrombic activity of the tested compound by oral administration, an animal model experiment was similarly performed, except that the intravenous injection is replaced with oral feeding at 1 hour prior to the electrical stimulation. A dosage amount of 4 mg/rat was administered. The result was shown in Figure 7C, which revealed that the significant antithrombic activity relative to the vehicle treated group was observed at 14, 15, 16, 17, 18, and 20 minutes.

What is claimed is:

1. A method for the screening of an extract of plant for biologically active ingredients, comprising fractionating a crude extract of plant on a solid support, adding a labeled target capable of binding to biologically active ingredients of interest in the crude extract onto the support, and then detecting and recovering the biologically active ingredients.

2. The method of claim 1, wherein the plant is a herb.

3. The method of claim 2, wherein the herb is *Carthamus tinctorius L.*

4. The method of claim 1, wherein the target is a protein.

5. The method of claim 4, wherein the protein is a glycoprotein.

6. The method of claim 5, wherein the glycoprotein is platelet membrane receptor protein gpIIb/IIIa.

7. The method of any one of claims 1 to 6, wherein one of the biologically active ingredients is a small molecule with molecular weight of 268 gm/mole.

8. The method of claim 7, wherein the small molecule is self-polymerizable.

9. The method of claim 7, wherein the small molecule exhibits an inhibition activity in platelet aggregation.

10. The method of claim 7, wherein the small molecule exhibits an *in vivo* antithrombic activity.

11. A kit for the screening of an extract of plant for biologically active ingredients, comprising a solid support and gridded components on the solid support that are fractionated from an extract of plant, and the reagents required for probe hybridization, washing and signal detection for selecting and recovering the biologically active ingredients.

12. The kit of claim 11, wherein the plant is a herb.

13. The kit of claim 12, the herb is *Carthamus tinctorius L.*

Abstract

A novel method for the screening of an extract of plant for biologically active ingredients is disclosed, which comprises allocating the components fractionated from a crude extract of plant on a gridded solid support, adding a labeled target as a probe onto the support and properly incubating the solid support, then detecting the expected signals and recovering biologically active ingredients corresponding to the signals. By applying the novel method, a biologically active small compound in a purified form from an extract of *Carthamus tinctorius L* that can specifically bind to a platelet membrane receptor protein gpIIb/IIIa is obtained.

Figure 1A

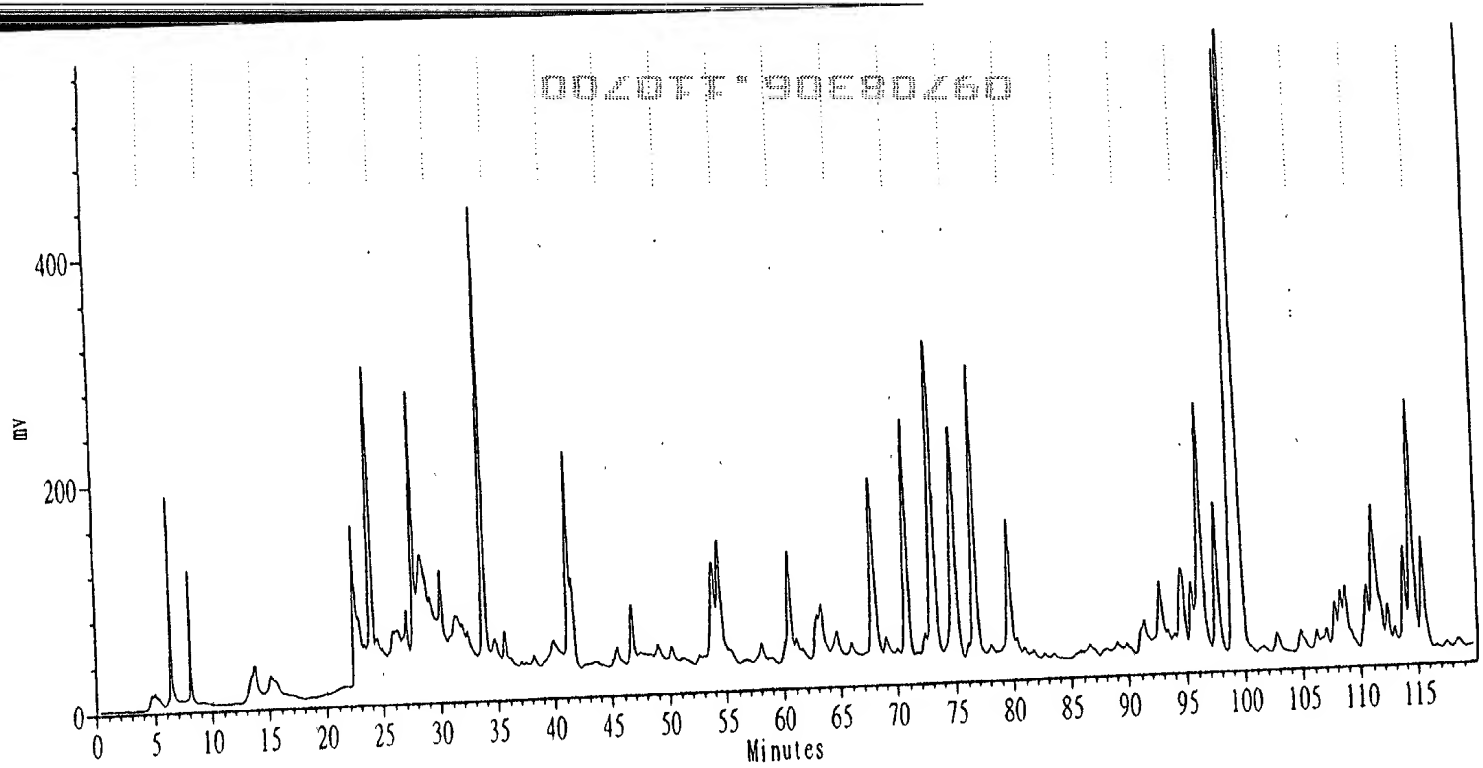


Figure 1B

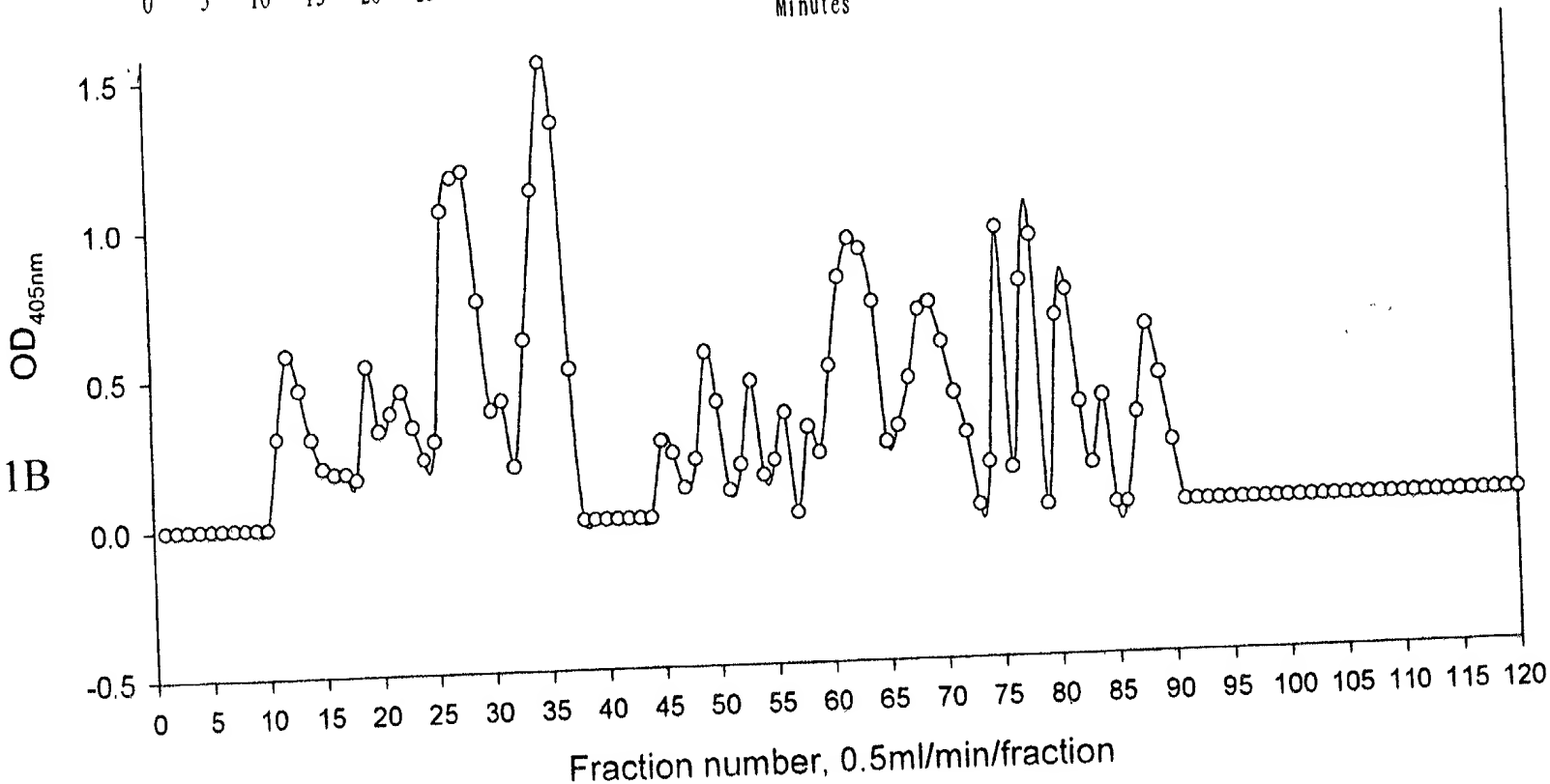


Figure 2A

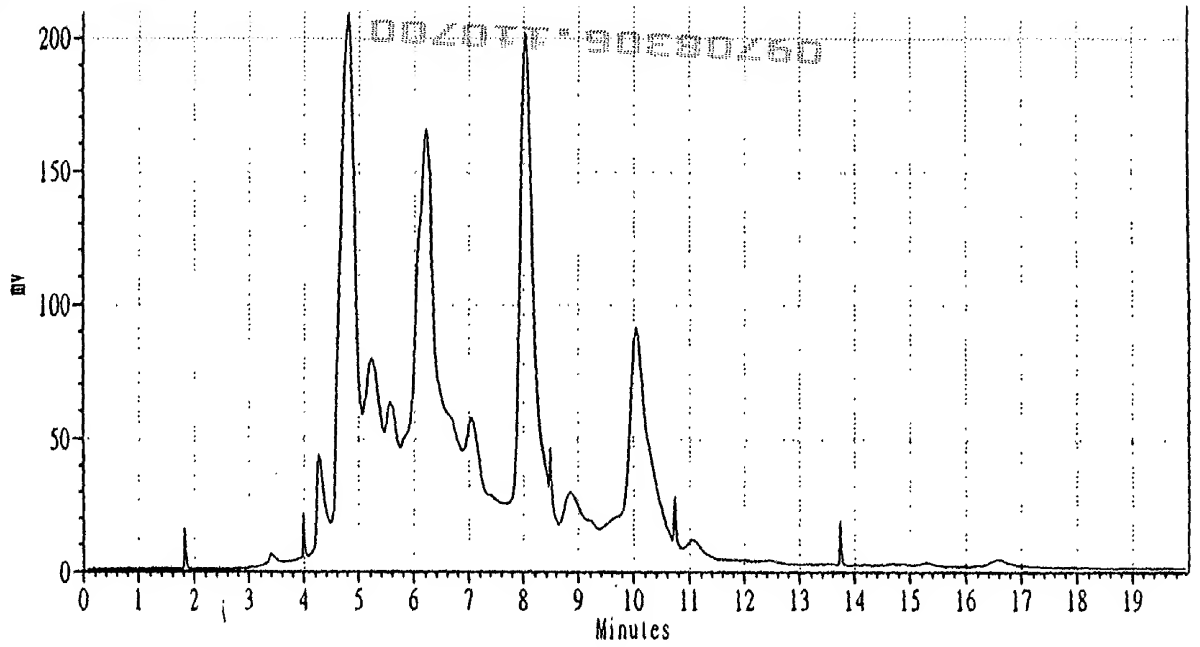
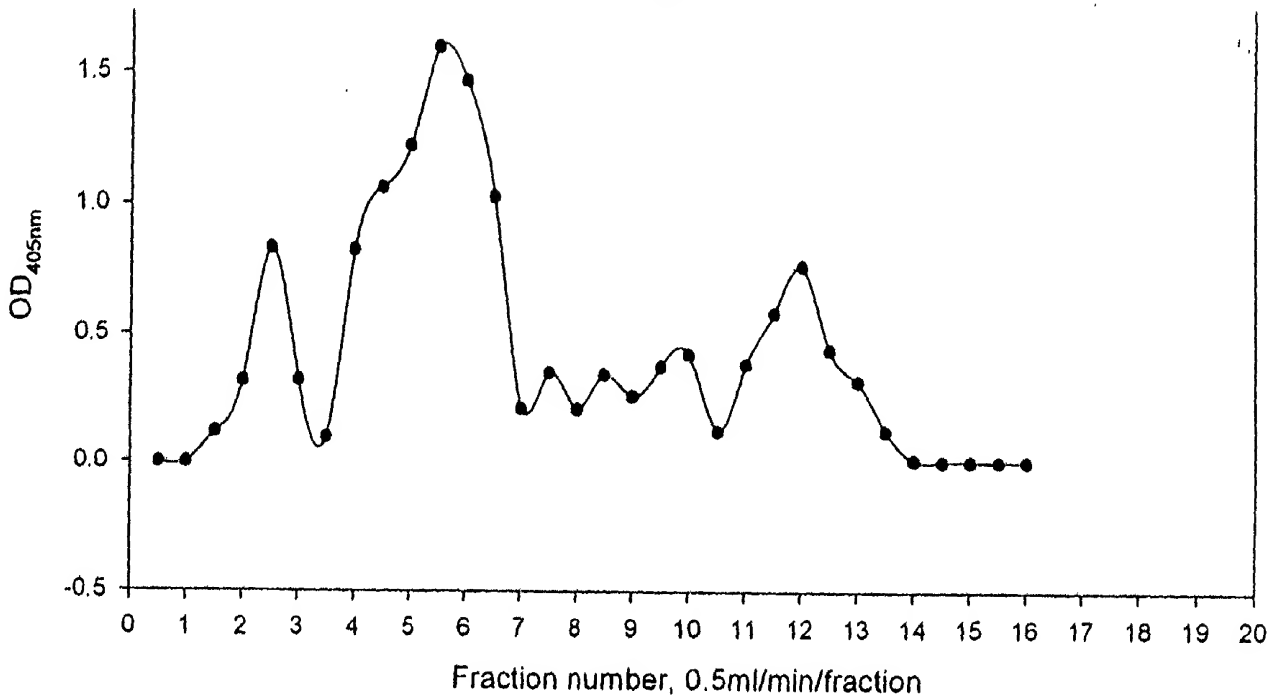


Figure 2B



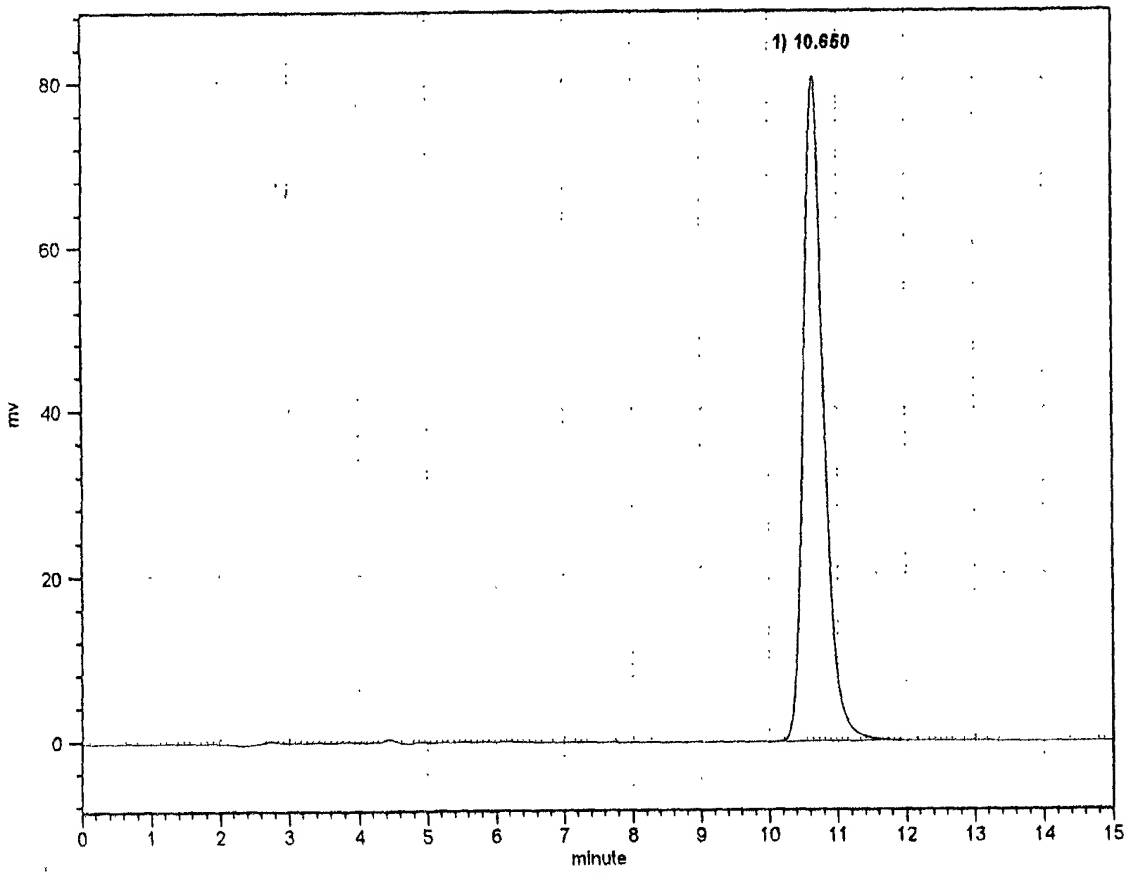


Figure 3

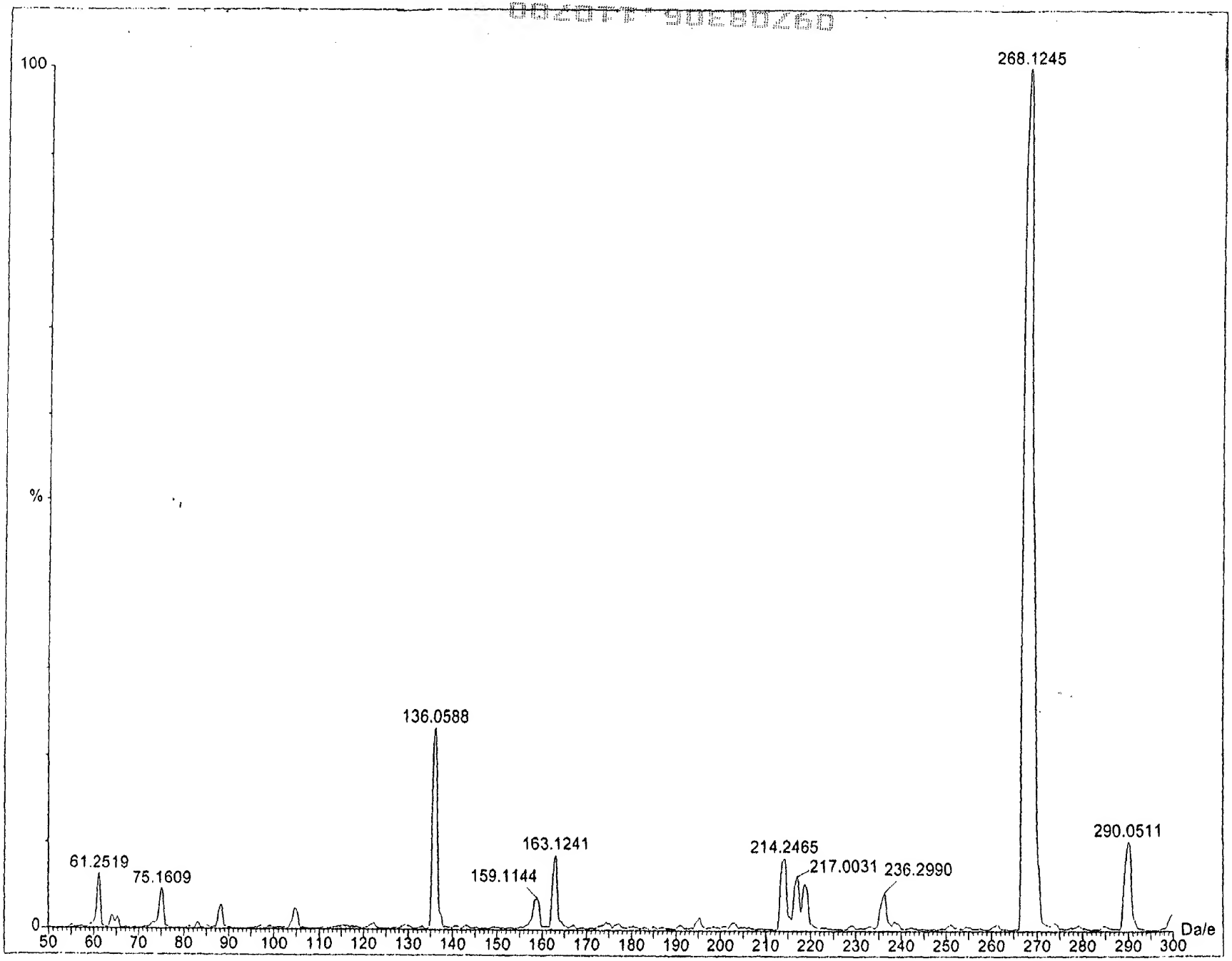

100
%
0
268.1245
136.0588
163.1241
159.1144
61.2519
75.1609
214.2465
217.0031
236.2990
290.0511
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300
Da/e

Figure 4

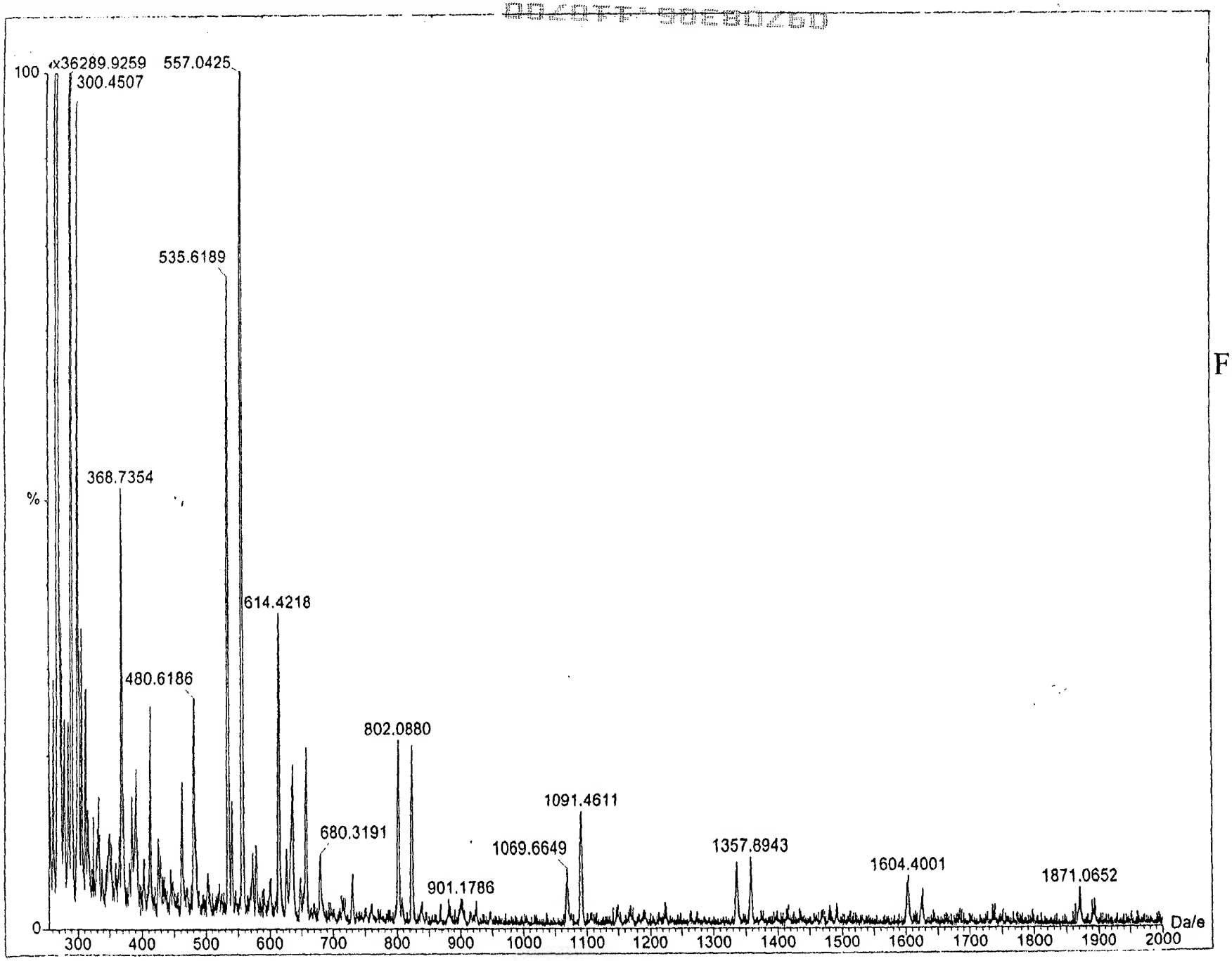
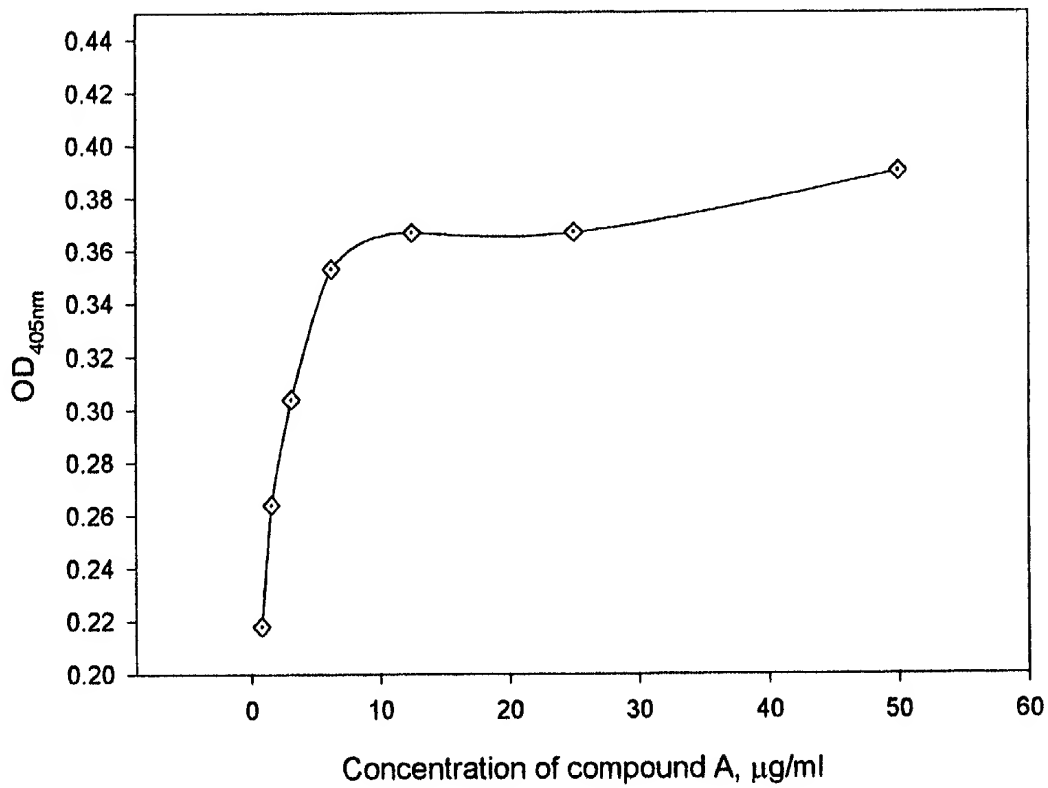

100
%
0
x36289.9259
300.4507
557.0425
535.6189
368.7354
614.4218
480.6186
802.0880
680.3191
1091.4611
1069.6649
901.1786
1357.8943
1604.4001
1871.0652
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
Da/e

Figure 4B

Figure 5


0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
OD405nm
0
10
20
30
40
50
60
Concentration of compound A, µg/ml

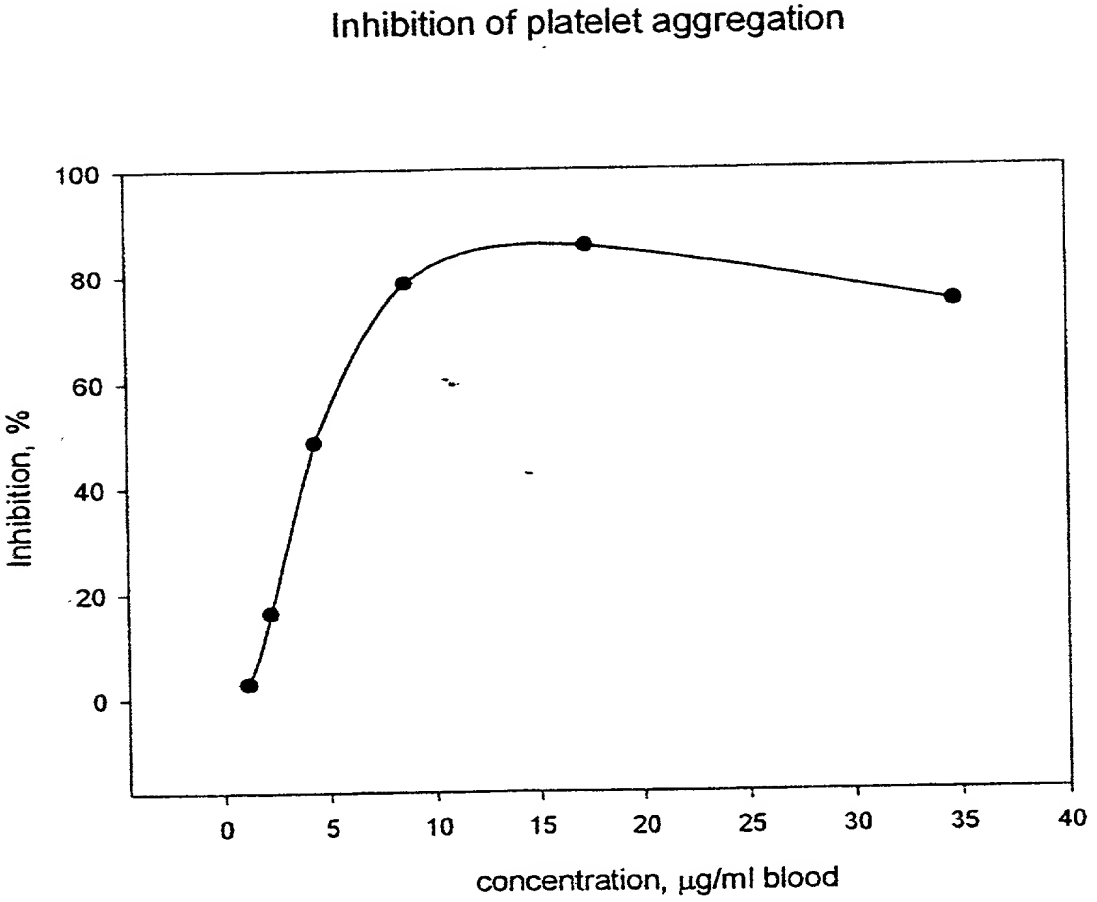

Inhibition of platelet aggregation
Inhibition, %
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
concentration, µg/ml blood

Figure 6A

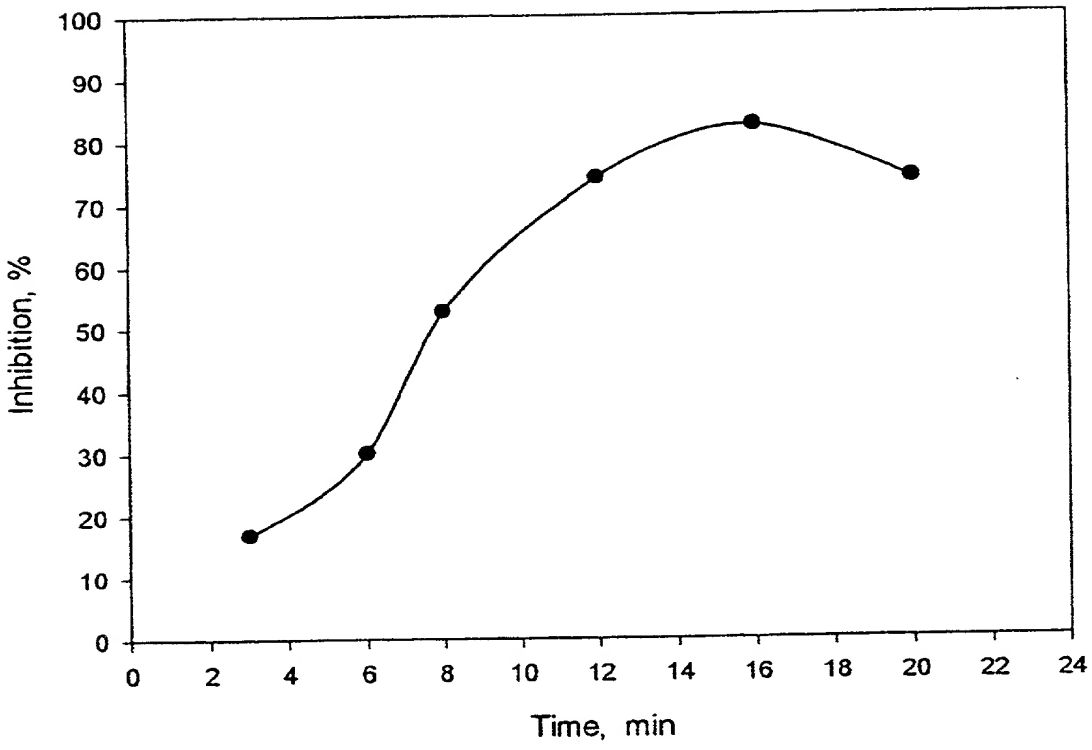

Time course of platelet aggregation inhibition
Inhibition, %
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time, min

Figure 6B

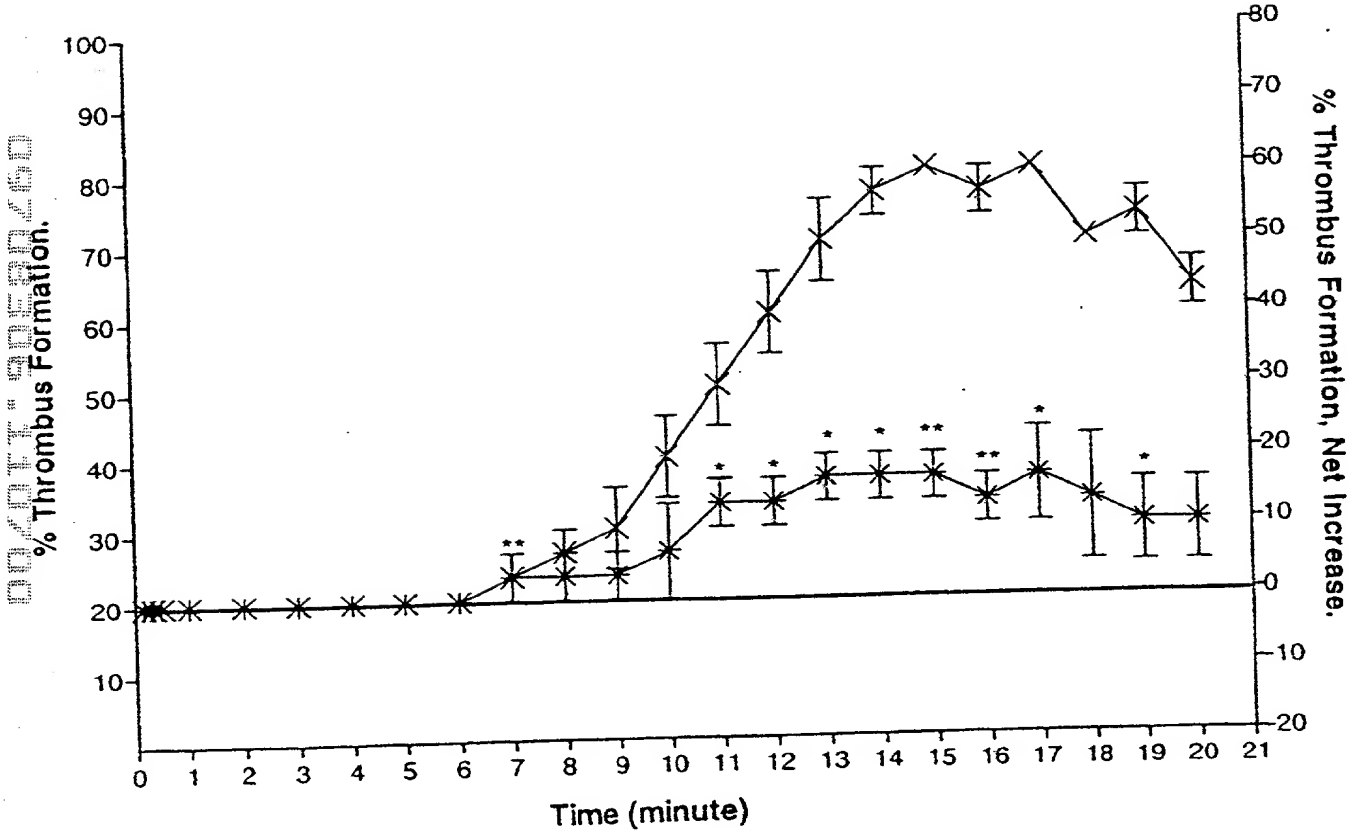

% Thrombus Formation.
% Thrombus Formation, Net Increase.
Time (minute)
100
90
80
70
60
50
40
30
20
10
80
70
60
50
40
30
20
10
0
10
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Figure 7A

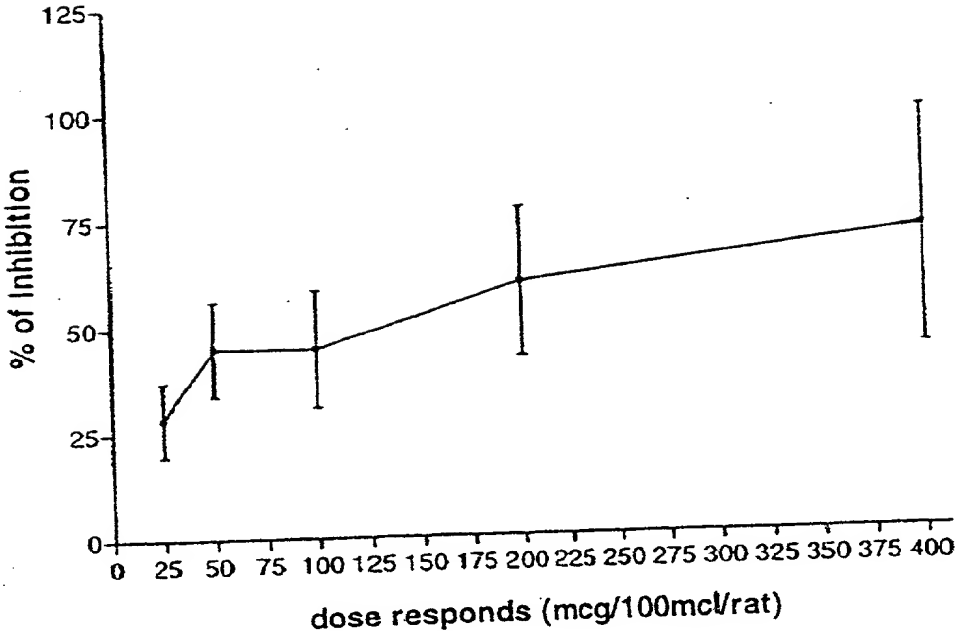

125
100
75
50
25
0
% of Inhibition
0 25 50 75 100 125 150 175 200 225 250 275 300 325 350 375 400
dose responds (mcg/100mcl/rat)

Figure 7B

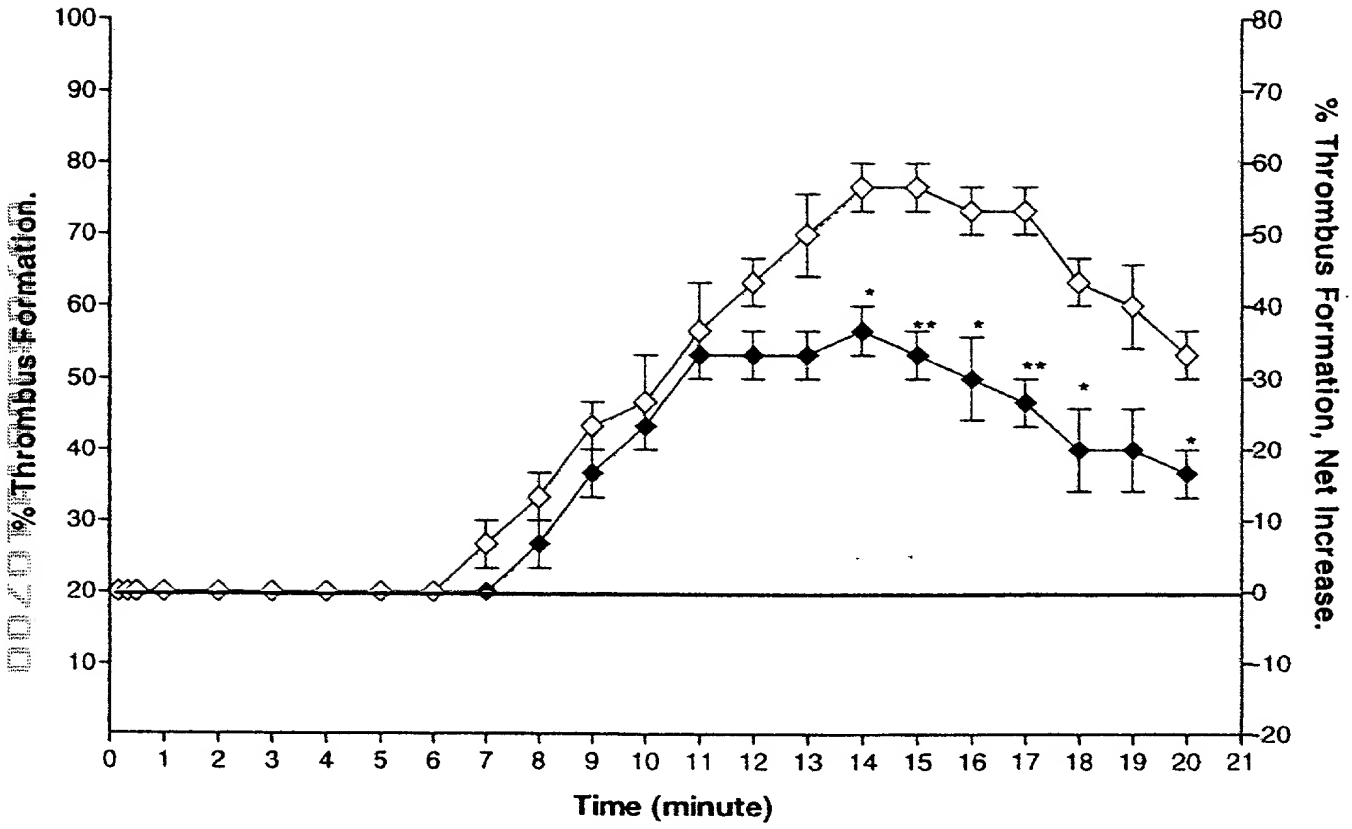

% Thrombus Formation.
% Thrombus Formation, Net Increase.
Time (minute)
100
90
80
70
60
50
40
30
20
10
80
70
60
50
40
30
20
10
0
-10
-20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Figure 7C

PATENT
Docket No.
205032000400

COMBINATION POWER OF ATTORNEY / DECLARATION FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTORS, WE HEREBY DECLARE THAT:

Our residence, post office address, and citizenship are as stated below next to our names.

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled: Novel method for the screening of extract of plant for active ingredients, the specification of which is attached hereto unless the following box is checked:

☐ was filed on * as United States Application Serial No. or PCT International Application No. * and was amended on * (if applicable).

WE HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

We acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

We hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority Claimed?
			☐Yes ☐No

We hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date

We hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, we acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status
		☐Patented ☐Pending ☐Abandoned

We hereby appoint the following attorneys and agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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